II. REMARKS

Applicants have amended the specification to correct various typographical and syntax errors. Applicants have also amended the specification to describe the present invention with greater particularity. The amendments introduced herewith find support in the original disclosure and thus do not constitute new matter.

For instance, paragraph 18 has been amended to recite "$GABA_B$ receptor 1" polypeptide and "$GABA_B$ receptor 2" polypeptide. These two terms are supported by the original SEQ ID NO. 2 and SEQ ID NO. 4. These two SEQ ID NOS. contain $GABA_B$ receptor 1 and 2 polypeptides, respectively (see also Figure 2). The limitation that the heterodimerization sequences are "linked to a cysteine residue" is also supported by the original SEQ ID NOS. 2 and 4; each SEQ ID NO. shows a $GABA_B$ polypeptide that is linked to a cysteine residue.

Paragraph 37 has been amended to describe the figure legend with greater particularity.

Amendments to the title at page 49 and paragraph 75 are merely typographical.

Paragraphs 105 and 106 have been amended to recite the term "essentially." This term is supported throughout the specification. In particular, paragraph 11 under the "Summary of the Invention" section describes that "*The subject antigen-binding unit is assembled and stabilized by the pairwise affinity of a distinct pair of heterodimerization sequences. The sequences are distinct in that at least one member of the heterodimerization pair is essentially incapable of forming homodimers under physiological buffer conditions and/or at physiological body temperatures.*"

Paragraph 110 has been amended to recite the specific findings reported in Kammerer et al. (1999) *Biochemistry* 38:13263-13269), copy of which is enclosed herewith for the Examiner's convenience. Like all references cited in the specification, Kammerer et al. is incorporated by reference in its entirety. Support for such incorporation is found at paragraph 16 of the original disclosure that states the following:

> "*Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure.*"

Paragraph 119 has been amended to recite "$GABA_B$ receptor 1" polypeptide and "$GABA_B$ receptor 2" polypeptide. These two terms are supported by the original SEQ ID NO. 2

and SEQ ID NO. 4. These two SEQ ID NOS. contain $GABA_B$ receptor 1 and 2 polypeptides, respectively (see also Figure 2). The limitation that the heterodimerization sequences are "linked to a cysteine residue" is also supported by the original SEQ ID NOS. 2 and 4; each SEQ ID NO. shows a $GABA_B$ polypeptide that is linked to a cysteine residue.

Paragraph 167 has been amended to describe the invention with greater particularity. The term transgene is replaced with "exogenous sequence."

Paragraph 201 has been amended to incorporate the results of Kammerer et al. *supra*.

New claims 87-93 have been added. No new matter is introduced in these newly added claims. Specifically, the term "growth factor receptors," "G-protein-coupled receptors," "neurotransmitters," and "nuclear hormone receptors" recited in claims 87-90, respectively, find support in the definition of "heterodimeric receptor" (see paragraph 84 at page 26 of the specification).

The recitation of "ccFv fragment" in claim 91 is supported by Figure 1 as well as the section entitled "Configurations and Modifications of Antigen-Binding Units (Abus)" at page 41.

The limitation of 37°C in claim 92 is supported by paragraph 109 at page 34, which states "*physiological body temperatures ranging from approximately room temperature to approximately 37°C.*"

The limitation that "first and second heterodimerization sequences are essentially incapable of forming homodimers when mixed in equimolar" in claim 93 is supported by the Kammerer disclosure, which is incorporated by reference.

Claim 1, 3, 8, 18, 19, 29, 30, 49, 51, 60, 67, 71 have been amended to more clearly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, claim 1, 3, 49, 51, 67, and 71 have been amended to recite the term fused "in-frame." Incorporation of this term is supported by the definition of "in-frame fusion" at paragraph 91 bridging pages 28 and 29. Additional support is found at paragraph 139 at page 48 that describes "*all nucleic acid sequences encoding the Abus are preferably assembled by in-frame fusion of coding sequences.*"

The recitation of "$GABA_B$ receptor 1" polypeptide and "$GABA_B$ receptor 2" polypeptide in claims 18-19, 29-30 is supported by SEQ ID NO. 2 and SEQ ID NO. 4 recited in the original claims. These two SEQ ID NOS. contain $GABA_B$ receptor 1 and 2 polypeptides, respectively

(see also Figure 2). The limitation that the heterodimerization sequences are "linked to a cysteine residue" is also supported by the original SEQ ID NOS. 2 and 4; each SEQ ID NO. shows a $GABA_B$ polypeptide that is linked to a cysteine residue.

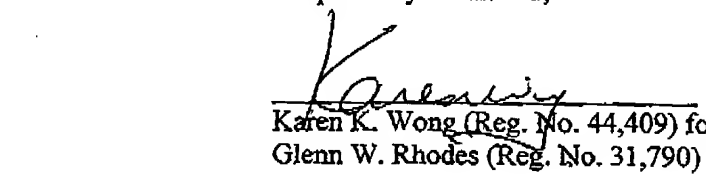
The term "essentially" incapable in claim 71 is supported throughout the specification. In particular, paragraph 11 under the "Summary of the Invention" section describes that "*The subject antigen-binding unit is assembled and stabilized by the pairwise affinity of a distinct pair of heterodimerization sequences. The sequences are distinct in that at least one member of the heterodimerization pair is essentially incapable of forming homodimers under physiological buffer conditions and/or at physiological body temperatures.*".

An issue of new matter is not raised by these amendments, and entry thereof is respectfully requested. Upon entry of this Amendment, claims 1-93 are now pending. The Examiner is invited to contact the undersigned at (650) 463-8100 with any questions, comments or suggestions relating to the above-identified patent application.

It is believed that sufficient fee is submitted herewith. However, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Arnold White & Durkee Deposit Account No. 01-508/13403.0004.NPUS00/RHO. Attached hereto is a copy of the "Version with Markings to Show Changes Made."

Dated: November 1, 2001

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph 18 has been amended as follows:

Preferred heterodimerization sequences contained in the subject antigen-binding units are derived from C-terminal sequences of $GABA_B$ receptor 1 and $GABA_B$ receptor 2, respectively. More preferably, the first heterodimerization sequence is linked to a cysteine residue, said first heterodimerization comprising ~~comprises~~ a $GABA_B$ receptor 1 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 2; and the second heterodimerization sequence is linked to a cysteine residue, said second heterodimerization comprising ~~comprises~~ a $GABA_B$ receptor 2 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4. Alternatively, the first heterodimerization sequence is linked to a cysteine residue, said first heterodimerization comprising ~~comprises~~ a $GABA_B$ receptor 2 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4; and the second heterodimerization sequence is linked to a cysteine residue, said second heterodimerization comprising a $GABA_B$ receptor 1 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 2.

Paragraph 37 has been amended as follows:

Figure 2 depicts the nucleotide and amino acid sequences of $GABA_B$ receptor 1 and 2 that were used in constructing the subject Abus. The coiled-coil sequences are derived from human $GABA_B$-R1 and $GABA_B$-R2 receptors. The coding amino acid sequences from $GABA_B$ receptor 1 begins with EEKS and ends with QLQS as shown in the top panel of Figure 2 ~~(SEQ ID NO. 2)~~. The coding amino acid sequences from $GABA_B$ receptor 2 begins with TSRL and ends with QLQD as shown in the bottom panel of Figure 2 ~~(SEQ ID NO.4)~~. A flexible SerArgGlyGlyGlyGly spacer was added to the amino-termini of R1 and R2 heterodimerization sequences to favor the formation of functional Fv heterodimer. To further stabilize the heterodimer, we have introduced a ValGlyGlyCys spacer to lock the heterodimeric coiled-coil pair via the disulfide bond between the cysteine residues (SEQ ID NOS. 2 and 4). The SerArg coding sequences at the N-terminus of the GGGG spacer provides XbaI or XhoI sites for fusion of the GR1 (heterodimerization sequence derived from $GABA_B$1 receptor) and GR2 (heterodimerization sequence derived from $GABA_B$2) domains to the carboxyl-termini of VH and VL fragments, respectively.

The title at page 49 has been amended as follows:

Polynucleotides, ~~and host cells~~ of the ~~present~~ Present ~~invention~~Invention

Paragraph 75 has been amended as follows:

"Single-chain antigen-binding unit" ("Sc Abu") refers to a monomeric Abu. Although the two domains of the Fv fragment are coded for by separate genes, a synthetic linker can be made that enables them to be made as a single protein chain (i.e. single chain Fv ("scFv") as described in Bird *et al.* (1988) *Science* 242:423-426 and Huston *et al.* (1988) *PNAS* 85:5879-5883) by recombinant methods. Other Sc Abus include antigen-binding molecules stabilized by the subject heterodimerization sequences (see e.g. Figure 18), and dAb fragments (Ward *et al.*, (1989) *Nature* 341:544-546) which consist of a VH domain and an isolated complimentarity determining region (CDR). An example of a linking peptide is $(GGGGS)_3$, which bridges approximately 3.5 nm between the carboxyl terminus of one V region and the amino terminus of another V region. Other linker sequences can also be used, and can provide additional functions, such as a means for attaching a drug or a solid support. A preferred single-chain antigen-binding unit contains VL and VH regions that are linked together and stabilized by a pair of subject heterodimerization sequences. The scFvs can be assembled in any order, for example, VH—(first heterodimerization sequence)-(second heterodimerization sequence) —VL, or V_L—(first heterodimerization sequence)-(second heterodimerization sequence) —VH.

Paragraph 105 has been amended as follows:

As noted above, proper assembly of polypeptide subunits to form a stable complex is required to ensure the biological function of a multimeric protein. Accordingly, a central aspect of the present invention is the design of a technique that enables specific assembly of selected monomeric polypeptides to effect efficient production of heteromultimers. The experimental design is particularly useful for generating and screening for heteromultimers such as Abus whose binding specificities depend on the assembly of specific subunits in a specific manner. Distinguished from the previously reported chimeric Abus, the subject Abus have one or more of the following unique features. First, the Abus are reconstititued via pairwise affinity of two heterodimerization sequences, at least one of which and preferably both of which, lack(s) detectable propensity to form homodimers. Unlike the previously reported dimerization sequences such as *Fos* and *Jun* leucine zippers that are known to form homodimers under both physiological buffer conditions and physiological body temperature (O'Shea *et al.* (1992) *Cell* 68: 699-708; Vidal *et al.* (1996) *Proc. Natl. Acad. Sci. U.S.A*), the subject heterodimerization sequences are essentially incapable of forming homodimers either under the specified buffer conditions and/or at the specified body temperatures. The subject heterodimerization sequences may be ~~further~~ also distinguished from the previously employed sequences at the structural level as detailed below.

Paragraph 106 has been amended as follows:

In one embodiment, the present invention provides a chimeric heteromultimer displayed on the surface of the host cell, wherein heteromultimer comprises: (i) a first polypeptide fused to

a first heterodimerization sequence and a surface presenting sequence; (ii) a second polypeptide fused to a second heterodimerization sequence; wherein the first and second polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences; wherein at least one of the heterodimerization sequences is essentially incapable of forming a homodimer under physiological buffer conditions and/or at physiological body temperatures.

Paragraph 110 has been amended as follows:

Second, the selected heterodimerization sequences must exhibit pairwise affinity resulting in predominant formation of heterodimers to a substantial exclusion of homodimers. Preferably, the predominant formation yields a heteromultimeric pool that contains at least 60% heterodimers, more preferably at least 80% heterodimers, more preferably between 85-90% heterodimers, and more preferably between 90-95% heterodimers, and even more preferably between 96-99% heterodimers that are allowed to form under physiological buffer conditions and/or physiological body temperatures. In certain embodiments of the present invention, at least one of the heterodimerization sequences employed to reconstitute an Abu is essentially incapable of forming a homodimer in a physiological buffer and/or at physiological body temperature. By "essentially incapable" is meant that the selected heterodimerization sequences when tested alone do not yield detectable amounts of homodimers in an *in vitro* sedimentation experiment as detailed in Kammerer *et al.* (1999) *Biochemistry* **38**: 13263-13269), or in the *in vivo* two-hybrid yeast analysis (see e.g. White *et al.* (*Nature* (1998) **396**: 679-682). Specifically, Kammerer et al. have demonstrated by sedimentation experiments that the heterodimerization sequences of $GABA_B$ receptor 1 and 2, when tested alone, sediment at the molecular mass of the monomer under physiological conditions and at physiological body temperatures (e.g. at 37^0C). When mixed in equimolar amounts, $GABA_B$ receptor 1 and 2 heterodimerization sequences sediment at the molecular mass corresponding to the heterodimer of the two sequences (see Table 1 of Kammerer et al.). In addition, individual heterodimerization sequences can be expressed in a host cell and the absence of homodimers in the host cell can be demonstrated by a variety of protein analyses including but not limited to SDS-PAGE, Western blot, and immunoprecipitation. The *in vitro* assays must be conducted under a physiological buffer conditions, and/or preferably at physiological body temperatures. Generally, a physiological buffer contains a physiological concentration of salt and at adjusted to a neutral pH ranging from about 6.5 to about 7.8, and preferably from about 7.0 to about 7.5. A variety of physiological buffers is listed in Sambrook *et al.* (1989) *supra* and hence is not detailed herein. Preferred physiological conditions are described in Kammerer *et al.*, *supra*.

Paragraph 119 has been amended as follows:

While a diverse variety of coiled coils involved in hetero-oligomerization can be employed in the subject invention, preferred coiled coils are derived from heterodimeric receptors. Accordingly, the present invention encompasses the coiled-coil dimeric sequences derived from $GABA_B$ receptors 1 and 2. In one aspect, the subject coiled coils comprise the C-terminal sequences of $GABA_B$ receptor 1 and $GABA_B$ receptor 2. In another aspect, the subject coiled coils are further linked to cysteine residues. The coiled coils are $GABA_B$ receptor 1 and 2

~~composed of two distinct~~ polypeptides of at least 30 amino acid residues, one of which is essentially identical to a linear sequence of comparable length depicted in SEQ ID NO. 2, and the other is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4.

Paragraph 167 has been amended as follows:

In constructing the subject vectors, the termination sequences associated with the ~~transgene~~ exogenous sequences are also inserted into the 3' end of the sequence desired to be transcribed to provide polyadenylation of the mRNA and/or transcriptional termination signal. The terminator sequence preferably contains one or more transcriptional termination sequences (such as polyadenylation sequences) and may also be lengthened by the inclusion of additional DNA sequence so as to further disrupt transcriptional read-through. Preferred terminator sequences (or termination sites) of the present invention have a gene that is followed by a transcription termination sequence, either its own termination sequence or a heterologous termination sequence. Examples of such termination sequences include stop codons coupled to various polyadenylation sequences that are known in the art, widely available, and exemplified below. Where the terminator comprises a gene, it can be advantageous to use a gene which encodes a detectable or selectable marker; thereby providing a means by which the presence and/or absence of the terminator sequence (and therefore the corresponding inactivation and/or activation of the transcription unit) can be detected and/or selected.

Paragraph 201 has been amended as follows:

Distinguished from previously characterized coiled-coil leucine zippers from the *Fos* and *Jun* proteins, the C-terminal coiled coil of $GABA_B$-R1 and $GABA_B$-R2 receptors do not form detectable homodimers under physiological conditions (e.g. *in vivo*); nor do they form homodimers at physiological body temperatures. Research by Kuner *et al.* and White *et al.* (*Science* (1999) 283: 74-77); *Nature* (1998) 396: 679-682)) have demonstrated the heterodimerization specificity of $GABA_B$-R1 and $GABA_B$-R2 *in vivo*. In fact, White *et al.* were able to clone $GABA_B$-R2 from yeast cells based on the exclusive specificity of this heterodimeric receptor pair. *In vitro* studies by Kammerer *et al. supra* has shown that neither $GABA_B$-R1 nor $GABA_B$-R2 C-terminal sequences is capable of forming homodimers in physiological buffer conditions when assayed at physiological body temperatures (see Table 1 of Kammerer). However, none of these researchers who were involved in the original isolation of the $GABA_B$-R2 gene and the characterization of the coiled-coil sequences describe or even suggest the use of this unique heterodimerization sequences for construction of heteromultimers such as antigen-binding units.

In the Claims:

The new claims 87-93 have been added:

87. (New). A non-single-chain antigen-binding unit of claim 1 or 3, wherein said first and second heterodimerization sequences comprise heterodimeric receptor sequences of growth factor receptors.

88. (New). A non-single-chain antigen-binding unit of claim 1 or 3, wherein said first and second heterodimerization sequences comprise heterodimeric receptor sequences of G-protein-coupled receptors.

89. (New). A non-single-chain antigen-binding unit of claim 1 or 3, wherein said first and second heterodimerization sequences comprise heterodimeric receptor sequences of neurotransmitters.

90. (New). A non-single-chain antigen-binding unit of claim 1 or 3, wherein said first and second heterodimerization sequences comprise heterodimeric receptor sequences of nuclear hormone receptors.

91. (New) A non-single-chain antigen-binding unit of claim 1 or 3, wherein the antigen-binding unit is a ccFv fragment.

92. (New) A non-single-chain antigen-binding unit of claim 1, wherein the physiological body temperatures are at about 37^0C.

93. (New) A non-single-chain antigen-binding unit of claim 1, wherein said first and second heterodimerization sequences are essentially incapable of forming homodimers when mixed in equimolar.

Claims 1, 3, 8, 18, 19, 29, 30, 49, 51, 60, 67, 71 have been amended as follows:

1. (Amended) A non-single-chain antigen-binding unit comprising:

(a) a light (L) chain polypeptide comprising a light (L) chain variable region fused in-frame to a first heterodimerization sequence;
(b) a heavy (H) chain polypeptide comprising a heavy (H) chain variable region fused in-frame to a second heterodimerization sequence;
wherein the L chain and the H chain polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences; and wherein at least one of the heterodimerization sequences is essentially incapable of forming a homodimer under physiological buffer conditions and/or at physiological body temperatures.

3. (Amended) A non-single-chain antigen-binding unit comprising:
(c) a light (L) chain polypeptide comprising a light (L) chain variable region fused in-frame to a first heterodimerization sequence;

(d) a heavy (H) chain polypeptide comprising a heavy (H) chain variable region fused in-frame to a second heterodimerization sequence;

wherein the L chain and the H chain polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences, said first and second heterodimerization sequences comprising heterodimeric receptor sequences that mediate heterodimerization of the receptors.

8. (Amended) The non-single-chain antigen-binding unit of claim 4, wherein both the first and the second heterodimerization sequences are linked to ~~contain~~ at least one cysteine residue.

18. (Amended) The non-single-chain antigen-binding unit of claim 4, wherein the first heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 1 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 2 and wherein the second heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 2 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4, wherein said first and second heterodimerization sequences are linked to cysteine residues.

19. (Amended) The non-single-chain antigen-binding unit of claim 4, wherein the first heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 1 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4; and wherein the second heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 2 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 2, wherein said first and second heterodimerization sequences are linked to cysteine residues.

29. (Amended) The single-chain antigen-binding unit of claim 23, wherein the first heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 1 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 2; and wherein the second heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 2 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4, wherein said first and second heterodimerization sequences are linked to cysteine residues.

30. (Amended) The single-chain antigen-binding unit of claim 23, wherein the first heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 1 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 4; and wherein the second

heterodimerization sequence is a heterodimerization sequence comprising a $GABA_B$ receptor 2 polypeptide of at least 30 amino acid residues that is essentially identical to a linear peptide sequence of comparable length depicted in SEQ ID NO. 2, wherein said first and second heterodimerization sequences are linked to cysteine residues.

49. (Amended) A method of producing a non-single-chain antigen-binding unit, comprising:
(a) expressing in a host cell a first recombinant polynucleotide encoding a light (L) chain polypeptide comprising a light (L) chain variable region fused in-frame to a first heterodimerization sequence, and a second recombinant polynucleotide encoding a heavy (H) chain polypeptide comprising a heavy (H) chain variable region fused in-frame to a second heterodimerization sequence; wherein the L chain and the H chain polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences; and wherein at least one of the heterodimerization sequences is essentially incapable of forming a homodimer under physiological buffer conditions and/or at physiological body temperatures; and optionally
(b) isolating the antigen-binding unit expressed in the host cell.

51. (Amended) A method of producing a non-single-chain antigen-binding unit, comprising:
(a) expressing in a host cell a first recombinant polynucleotide encoding a light (L) chain polypeptide comprising a light (L) chain variable region fused in-frame to a first heterodimerization sequence, and a second recombinant polynucleotide encoding a heavy (H) chain polypeptide comprising a heavy (H) chain variable region fused in-frame to a second heterodimerization sequence; wherein the L chain and the H chain polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences, said first and second heterodimerization sequences comprising heterodimeric receptor sequences that mediate heterodimerization of the receptors; and optionally
(b) isolating the antigen-binding unit expressed in the host cell.

60. (Amended) The method of claim 56, wherein both the first and the second heterodimerization sequences are linked to ~~contain~~ at least one cysteine residue.

67. (Amended) A method of producing a non-single-chain antigen-binding unit, comprising:
(a) preparing a first recombinant polynucleotide encoding a light (L) chain polypeptide comprising a light (L) chain variable region fused in-frame to a first heterodimerization sequence, and a second recombinant polynucleotide encoding a heavy (H) chain polypeptide comprising a heavy (H) chain variable region fused in-frame to a second heterodimerization sequence; wherein the L chain and the H chain polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences; and wherein at least one of the heterodimerization sequences is essentially incapable of forming a homodimer under physiological buffer conditions and/or at physiological body temperatures; and
(b) allowing the first and second polypeptides to dimerize via pairwise affinity of the first and second heterodimerization sequences.

71. (Amended) A method of displaying a chimeric heteromultimer comprising at least two polypeptides on a surface of a host cell, the method comprising:
expressing in the host cell
(a) a first recombinant polynucleotide encoding a first polypeptide fused in-frame to a first heterodimerization sequence and a surface presenting sequence;
(b) a second recombinant polynucleotide encoding a second polypeptide fused in-frame to a second heterodimerization sequence;
wherein the first and second polypeptides dimerize via pairwise affinity of the first and second heterodimerization sequences; wherein at least one of the heterodimerization sequences is essentially incapable of forming a homodimer under physiological buffer conditions and/or at physiological body temperatures.